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Assessment of levels and distribution of progesterone in receiving waters and wastewaters in the vicinity of Arusha city,Tanzania

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NM-AIST

ASSESSMENT OF LEVELS AND DISTRIBUTION OF PROGESTERONE IN RECEIVING WATERS AND WASTEWATERS IN THE VICINITY OF ARUSHA CITY, TANZANIA

Hildegard R. Kasambala

Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Environmental Science and Engineering of the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania.

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ABSTRACT

This study aimed at investigating the levels and distribution of progesterone in receiving waters and wastewaters in Arusha, a fast-growing urban area and third largest city in Tanzania. Also, intend to assess the efficiency of waste stabilisation ponds (WSP) and constructed wetland (CW) in removing progesterone. The study was conducted along the Themi River, (WSPs) and CW. Progesterone was detected and quantified by using an Enzyme-Linked Immunosorbent Assay (ELISA) kit. For Themi River samples, the level of progesterone obtained ranged from '*no detection*' to 439.00 ng/L with a mean value of 120.30 ng/L. The levels detected were significantly higher in the midstream three times and seven times than upstream and downstream, respectively ($P < 0.05$). Progesterone was spatially distributed much at midstream than upstream and downstream. The elevated values at midstream were attributable to livestock, WSPs and household effluents; agricultural activities; and sewage infiltration. WSPs were observed to release 215 ng/L of progesterone at effluent with zero progesterone detected at the effluent from CW with a removal efficiency of 75% and 100% for WSP and CW, respectively. Although progesterone removal efficiency was high, the amount released was still high enough to cause harm to aquatic organisms. Progesterone levels in the present study, although not extremely high, correspond to those associated with harmful effects in other studies. Therefore, this study suggests that anthropogenic activities conducted nearby the rivers should be strictly avoided to reduce the amount of progesterone to be released to the river.

DECLARATION

I, Hildegard R. Kasambala, do at this moment declare to the Senate of the Nelson Mandela African Institution of Science and Technology that, this is my original work and that it has neither been Submitted nor being simultaneously submitted for degree award in any other institution.

Hildegard R. Kasambala ______________ 31 / 12 / 2019

Name and signature of candidate Date

The above declaration is confirmed

Dr. Mwemezi J. Rwiza ______________ 31 December 2019

Name and signature of supervisor (1) Date

Prof. Robinson H. Mdegela ______________ 31 December 2019

Name and signature of supervisor (2) Date

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Dr. Mwemezi J Rwiza   Date

31 December 2019

Prof. Robinson H. Mdegela   Date

CERTIFICATION

The undersigned prove that they have read and confirmed that, at this moment, recommend for the examination of the dissertation. They authorize; Assessment of levels and distribution of progesterone in receiving waters and wastewaters in the vicinity of Arusha city, Tanzania to be accepted in partial fulfilment of the requirements for the Degree of Master of Environmental Science and Engineering of the Nelson Mandela African Institution of Science and Technology Arusha, Tanzania.

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Dr. Mwemezi J. Rwiza

(Principal supervisor)

Date: 31/12/2019

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DEDICATION

I want to dedicate this work to my lovely husband Beda Pius Mtweve, who has always believed in me and provided constant encouragement to make through the University life — I love you, sweetheart. This also goes to my three adorable daughters: Careen, Maureen and Beatrice for their persistence and obedience during my long absence. Thank you, girls.

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LIST OF ABBREVIATIONS AND SYMBOLS

17α-OHP	17α-Hydroxyprogesterone
AfDB	African Development Bank
AUWAS	Arusha Urban Water and Sanitation Authority
BFRs	Brominates Flame Retardants
DDT	Dichlorodiphenyltrichloroethane
DO	Dissolved oxygen
EDCs	Endocrine Disrupting Compounds
ELISA	Enzymes-linked Immunosorbent Assay
GPS	Global Positioning System
MEWES	Material, Energy, Water and Environmental Sciences
MUST	Mbeya University of Science and Technology
MSc	Master of Science
NM-AIST	The Nelson Mandela African Institution of Science and Technology
PCBs	Polychlorinated biphenyls
SPE	Solid Phase Extraction
TBS	Tanzania Bureau of Standards
TDS	Total dissolved solids
UNEP	United Nations Environment Programme
USA	United States of America
WESE	Water Environmental Science and Engineering

WHO	World Health Organization
WSPs	Waste Stabilization Ponds

CHAPTER ONE

INTRODUCTION

1.1 Background

Globally, numerous environmental chemicals are categorised as endocrine-disrupting compounds (EDCs) (WHO, 2012). EDCs refer to any exogenous compound capable of interfering with the normal functioning of natural hormones (Zoeller *et al.*, 2012). These compounds are so-named due to their ability to interrupt with natural hormonal activities in the body resulting in increased disease vulnerability in organisms, including prostate cancer (Bornman *et al.*, 2017). EDCs can directly interact with endogenous hormonal receptors and mimic their normal functioning, either synergistically or antagonistically (Tyler, Jobling & Sumpter, 1998). Exposure to an aquatic environment contaminated with EDCs can have adverse effects on the nervous, immunity, reproductive, and urogenital systems of both human and marine organisms. Moreover, human exposure to EDCs can lead to infertility, abnormal prenatal, as well as abnormalities in child development (Mitani, Fujioka & Kataoka, 2005). EDCs exposure effects can be transferred from one generation to another (Anway, Cupp, Uzumcu & Skinner, 2005). According to Crews *et al.* (2007), EDCs' effect is not only transferred within a generation but also trans- population. Generally, the impact of the environmental pollutant in most developing countries is high due to lack of knowledge and abilities to deal with pollutant.

Several EDCs have been studied worldwide; these include PCB's, dioxins, nonylphenol, Bisphenol A, estrogen, dichlorodiphenyltrichloroethane (DDT), pesticides, and progestogens. Most of these studies focused much on the quantification and effects of other EDCs, e.g. estrogen in the receiving rivers (Msigala, Mabiki, Styrishave & Mdegela, 2017; Leusch *et al.*, 2018) However, in developing countries like Tanzania, there is limited information on the levels and distribution of EDCs with many studies in heavy metals (Mdegela *et al.*, 2009; Rwiza, Oh, Kim & Kim, 2018). Even globally, research coverage on progesterone levels in aquatic systems is limited. Progesterone is one of the EDCs, which is sometimes categorised as progestagens or gestagens (King & Brucker, 2010). Progesterone is among the steroid EDCs produced highly by both female and male human body to help maintain pregnancy, regulate gamete maturation, organise reproductive behaviour, sperm capaciting and influence spermatogenesis (WHO, 2012; Bergman *et al.*, 2013). It also acts as circulating hormones and secreting pheromones (Norris & Carr, 2013; Orlando & Ellestad, 2014). Moreover, progesterone steroid hormones also bind and activate the progesterone receptor (Harvey, Clark, Finkel, Rey & Whalen, 2011). Progesterone is one of the natives and major gestagens in the body which are widely used in medication such as oral contraceptives and hormone replacement therapeutics like menopause, hypocrinism and transgender (Harvey *et al.*, 2011).

Wastewater effluents and agricultural runoff effluents which are rich in progesterone can cause abnormalities in fishes' behaviours, sperm motility, neuroendocrine effect and gene expression in gonads (DeQuattro *et al.*, 2012; Orlando & Ellestad, 2014). Surface water is mostly polluted by progesterone through wastewater, paper mill, agricultural runoff and livestock effluents. Progestonic entities are commonly found in large quantities in aquatic environments because they are excreted through urine in significant amounts by a human; administered to animals as growth promoters and excreted by animals as endogenous hormones. Moreover, progesterone contributed by natural degradation of plant leaves present in the aquatic environment (Jenkins, Wilson, Angus, Howell & Kirk, 2003), the reason why sometimes progesterone may be found even in places with no anthropogenic activities.

Other studies on progesterone have covered sources and effects of progesterone, especially about surface water, with little or no focus on the levels and distribution of progesterone in receiving rivers (DeQuattro *et al.*, 2012). This study focuses on the distribution and levels of progesterone in its potential source, the WSPs, as well as the levels and distribution of progesterone in a corresponding receiving water body. Flowing across the eastern part Arusha city, Themi River receives municipal wastewater effluents; untreated sewage effluents from industries and residences; livestock effluents as well as agricultural effluents. Anthropogenic pollution in Themi River is known to cause harm to the downstream aquatic environment and human health (Senzia *et al.*, 2009; Lyimo, 2012). Other sources of progesterone in Themi River may include municipal wastewater, paper mill effluents, agricultural runoff and livestock effluents. It is known that most of African WSPs are less efficient in removing EDCs (Madikizela, Tavengwa & Chimuka, 2017), thus pollution the receiving. Therefore, this study intended to assess the levels and distribution of progesterone in Themi River and how the WSPs and CW contribute to progesterone pollution in the river. Furthermore, the study was aiming to identify the efficiency of WSPs and CW in removing progesterone EDCs.

1.2 Problem statement and justification

Several studies have been carried out concerning with EDCs, but the global rate and extent of EDCs pollution are increasing. Most studies explored much on the effect of progesterone on the aquatic organisms and its occurrence to the marine environment (Chang, Wan, Wu, Fan & Hu, 2011; DeQuattro *et al.*, 2012). However, little information recognised about the levels and distribution of progesterone in receiving and wastewaters of many sub-Saharan countries. Therefore, the proposed study intends to bridge this knowledge gap by assessing the levels of progesterone in receiving river, CW and WSP. Also, identify how progesterone distributed along the Themi River as the receiving river. Also, the study asses the effectiveness of WSPs and constructed CW in removing progesterone. Therefore, information from this study will bring awareness to the communities in African countries since many receiving rivers polluted due to the inefficiency of conventional wastewater treatment and poor control of anthropogenic activities.

1.3 Research objectives

1.3.1 General objective

To assess the levels and distribution of progesterone disrupting compounds in receiving waters, WSP and CW of Arusha, Tanzania.

1.3.2 Specific objectives

i. To quantify the levels of progesterone in the Themi River, WSPs and constructed wetland.
ii. To determine the spatial distribution and origin of progesterone in the Themi River of Arusha.
iii. To evaluate the efficiency of WSPs and constructed wetland in removing progesterone from wastewater.

1.3.3 Research questions

i. What are the levels of progesterone hormone in the Themi River, constructed wetland and WSP of Arusha?
ii. To what extent do the progesterone steroid hormone are spatially distributed to the Themi River of Arusha city?

iii. What is the efficiency of WSPs and constructed wetland in removing progesterone EDCs?

1.4 Significance of the study

The levels and distribution of hormonal pollutants in the surface waters in growing urban places in developing countries are highly understudied. Arusha city endowed with many rivers including Themi River, which is among the significant sources of water for agricultural uses, domestic and industrial uses. The status of progesterone enrichment in a river that passes through an urban area will bring awareness to the community on what anthropogenic activities contribute to the level and distribution of progesterone in receiving stream. This information will generate the habit of practicing sanitation principles at the individual level and different institutions in general. Also, the efficiency of WSPs and CW in removing progesterone in wastewaters assessed, provide baseline information on the preparation of guidelines of the allowable amount of progesterone to be released to the receiving water for the benefit of both the aquatic environment and human health in general.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

Varieties of chemical compounds when introduced to the aquatic natural environment may disrupt the function of natural endocrine hormones (Bergman *et al.*, 2013). The presence of these EDCs and other micropollutants in the environmental waters affects the aquatic and terrestrial organisms (Bergman *et al.*, 2013; Sun, Wang & Zhou, 2013; Leusch *et al.*, 2018). The Exposure of living organisms to EDCs may lead to various health problems, including cancers in male and female organisms, reproduction problems, obesity and diabetes (Qiang, Dong, Zhu, Qu & Nie, 2013; Gore *et al.*, 2014).

Humans and other living organisms may be exposed to EDCs in different ways including: food and water consumption, skin contact and breathing the contaminated air, transfer from mother to fetus or mother to newborn during breastfeeding (Diamanti-Kandarakis *et al.*, 2009; Gore *et al.*, 2014). Organisms at high trophic levels can also be contaminated through the consumption of lower trophic level organisms, which have consumed on EDC-containing materials (Gore *et al.*, 2014). The most susceptible groups of people to EDCs are those who work mostly with pesticides, fungicides, industrial chemicals, contaminated office spaces, and children toys (Diamanti-Kandarakis *et al.*, 2009).

2.2 Sources and distribution of progesterone in rivers

Different studies have been carried out showing the sources and distribution of EDCs in the aquatic environment, including river water, spring water, lakes, and wastewater effluent (Manickum & John, 2014; Msigala *et al.*, 2017; Damkjaer, Weisser, Msigala, Mdegela & Styrishave, 2018). Highly industrial areas in the world are suspected to be more contaminated by a wide range of micropollutants from industrial chemicals that leach into the soil and pollute ground and surface waters (Diamanti-Kandarakis *et al.*, 2009). According to Kuster, de Alda &Barceló (2004), progesterone is easily distributed in the environment and expected to accumulate in river sediments and soils for a long time. Progesterone may also be contributed by the municipal, hospital and industrial wastewater effluents (Chang, Wan & Hu, 2009). Others sources may include agricultural runoff, livestock feedlot effluent from manure and sewage sludge which usually enter the aquatic environment through receiving rivers (Jenkins *et al.*, 2003; Chang *et al.*, 2011; Orlando & Ellestad, 2014). Progesterone distribution may be understood from the comparison between wastewater effluents and receiving rivers downstream. According to Jenkins *et al.* (2003), paper mill industries contribute much to the progesterone distribution because in their study they found that the concentration of progesterone in effluents downstream of the industries was higher compared to upstream levels.

It has been observed that progesterone concentration decreases along the stream as water moves downstream because it undergoes biotransformation, degradation, adsorption, and mineralization into other androgen-like components (Durhan *et al.*, 2002; Jenkins *et al.*, 2004). Androgen present in river water usually acts as EDCs and is known to lead into emasculation of mosquitoes at downstream area of pulp mill effluent (Jenkins *et al.*, 2001; Durhan *et al.*, 2002; Jenkins *et al.*, 2003). However, there is still lack of adequate information on the spatial distribution of progesterone in receiving water bodies making this a study of a great need.

2.3 Effects of progesterone exposure to living organisms

Different studies have indicated that excessive exposure of living organism to EDCs may cause various health effects including male infertility, declines in male offspring, abnormalities in male reproductive organs, female sterility, early puberty in girls as well as ovarian and other cancers (DeQuattro *et al.*, 2012; Kabir, Rahman & Rahman, 2015). Other effects caused by EDCs such as neurone disorder are more prominent to young ones than to the adults and are known to be transmittable to more than one generation (Anway *et al.*, 2005; Crews *et al.*, 2007). Most EDCs from industrial areas leache into the soils and are then uptaken by plants and organisms of lower trophic levels such and cause higher trophic level organisms to be more affected through bioconcentration (Diamanti-Kandarakis *et al.*, 2009; Wojnarowicz, 2013). Increased cases of breast cancer in more industrialized countries may be due to high exposure to EDCs (Diamanti-Kandarakis *et al.*, 2009).

There have been reports on the decrease in fertility rates, fecundity as well as significantly reduced gonadosomatic index, vitellogenin gene expression; also, hermaphroditism in fish linked to high levels of EDCs in the aquatic environs (Zeilinger *et al.*, 2009; DeQuattro *et al.*, 2012). Moreover, high environmental concentration of progesterone can affect the fecundity in fathead minnow with no effect on embryonic development (DeQuattro *et al.*, 2012). EDCs are known to affect human health in different ways. According to Bergman *et al.* (2013), nearly

40% of men in some countries experience poor semen quality that could be linked to the effects of EDCs. Impacts of EDCs depend on its concentration in the environment, increase in persistence, bioaccumulation, time of being exposed, and the way of biotransformation and its removal from the environment (Esplugas, Bila, Krause & Dezotti, 2007). Therefore, results from this study will provide useful information to the community to help reduce the risks associated with high-level exposure to environmental EDCs.

2.4 Levels of EDCs in the aquatic environment

Studies related to levels of EDCs in many parts of the world have been carried by many scholars. Transport means of many known and potential EDCs is through natural processes as well as through commerce, leading to worldwide exposure (Le, 2012; Havens *et al.*, 2014). Progesterone has been detected in receiving rivers and wastewater effluent (Manickum & John, 2014; Payus, John, Wan, Hsiang & Kui, 2016). Receiving streams are known to have progesterone levels of ranging from 5 to 199 ng/L (Kolpin *et al.*, 2002; Chang *et al.*, 2009). About 2059 ng/L and 440 ng/L of progesterone where identified in River Fenholloway at Taylor, Florida (Jenkins *et al.*, 2001; Jenkins *et al.*, 2003). The highest level of progesterone of 3.112 ng/mL (6741 ng/L) was detected in Malaysia receiving rivers (Payus *et al.*, 2016). Various levels of progesterone have been detected in wastewaters including 408 ng/L (Manickum & John, 2014) and 904 ng/L (Madikizela *et al.*, 2017) found in South African waters, 108 ± 89 ng/L in Beijing, China (Chang *et al.*, 2011) and 260 ng/L in Wetzikon city, Switzerland (Zhang, Zhao & Fent, 2017). However, the highest level of progesterone of 16.687 ± 6.233 ng /mL (16687 ng/L) reported in Malaysia (Payus *et al.*, 2016) with the smallest concentration of 2.5 ng/L identified in Belgium (Pauwels, Noppe, De Brabander & Verstraete, 2008). Other studies show that livestock effluents contribute to the progesterone in receiving environment in which 375 ng/L detected in snowmelt associated with livestock operation in Wisconsins (DeQuattro *et al.*, 2012; Havens *et al.*, 2014). Progesterone concentration of 3470 ± 121 ng/L reported from swine and dairy cattle farms, China. There is limited information on the levels of progesterone from upstream to downstream of the receiving waters. This calls for studies that examine the concentrations and spatial distribution of endocrine disrupting progesterone at different river sections.

2.5 Efficiency of WSPs and constructed wetlands in removing EDCs

WSPs and CW systems do utilize natural processes in eliminating different types of pollutants. Constructed wetlands with horizontal sub-surface flow have been effectively used for treating numerous kinds of wastewater for several years. Most such systems are usually planned to treat municipal sewers reused for different activities, including agricultural and industrial applications (Vymazal & Kröpfelová, 2009).

Methods involved in removing pollutants in wetland systems include vegetation, soils, and their associated microbial assemblages (Vymazal, 2014; Vymazal, Březinová & Koželuh, 2015). The removal of contaminants in the constructed wetland and WSPs systems is complicated and usually influenced by varieties of removal mechanisms, including sedimentation, filtration, precipitation, volatilization, adsorption, plant uptake, and various microbial processes (Faulwetter *et al.*, 2009). These processes, directly and indirectly, are influenced by loading rates, temperatures, and soil types, operational strategies and redox conditions in the wetland bed (Stein, 2003). The efficiency of EDCs removal in WSPs depend on pond design, reduced pond repairs and physical-chemical parameters such as pond surface area, water depth, solution pH and dissolved oxygen (Msigala *et al.*, 2017). However, WSPs and CW systems have not widely been used due to lack of awareness and indigenous knowledge in developing the technology (Vymazal, 2009).

WSPs play a significant role in reducing the concentration of EDCs discharged from municipal and industrial wastewaters. A study on the role of wetlands in pollutant removal showed the various percentage efficiencies of WSPs in removing EDCs with 92% for the overall performance and 98% for removing progesterone (Manickum & John, 2014). Another study showed high efficiency in eliminating different types of steroids include the overall performance of 52 ± 32% and 94 ± 8% (Damkjaer *et al.*, 2018).Vymazal *et al.* (2015), showed that constructed wetland with a subsurface flow plays a more significant role in removing different kinds of steroids from wastewater. Lack of standard guidelines for the allowable amount of progesterone safe for the human health and aquatic microorganism makes the present study very valuable.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site description

This study was carried out along the Themi River and the Arusha municipal WSPs in the northeastern part of Tanzania (see Fig. 1 and Fig. 2). Themi River has a length of 46.41 kilometers and serves a population of 416,442 (NBS, 2012). It lays between latitudes 2^0 and 6^0 South and 34.5^0 and 38^o East of the Greenwich. Themi River originates from the Mount Meru slopes at Olgilai village about 1700 meters above the sea level and flows through Arusha city at an altitude of 1254 m (Lyimo, 2012). Human activities taking place along the river include agriculture, livestock keeping, industrial activities, car washing, bathing, washing of clothes, etc.

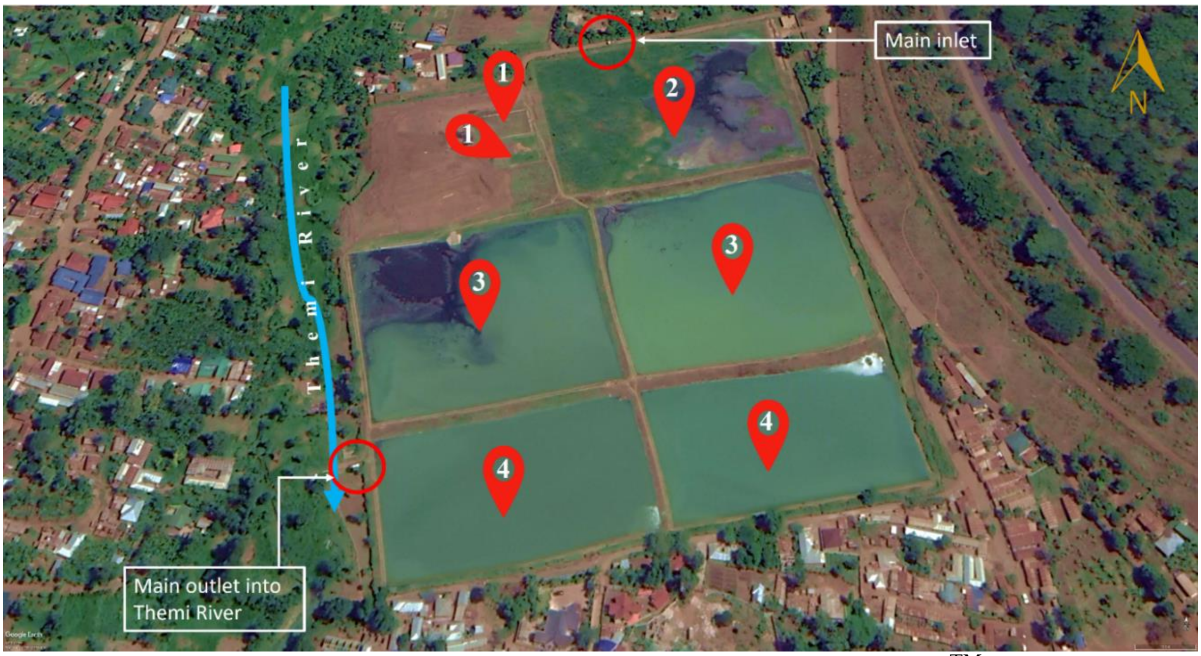

Figure 1. Layout of Arusha waste stabilisation ponds from Google Earth^{TM}: Blue arrow line on the left indicates the direction of flow of Themi River. Placeholder 1 = Domestic wastewater disposal tanks, 2 = Anaerobic pond, 3 = Facultative ponds, and 4 = Maturation ponds.

Themi River and the associated WSPs were selected as sampling sites because of the anthropogenic activities taking place within the area which have high chances of producing effluents with progesterone. The control samples collected from the river source in Machame sub-village of Olgilai area at the foot of Mount Meru. This river source is free from anthropogenic activities. The area used as a catchment point for the Arusha Urban Water and Sanitation Authority (AUWSA). Along the river, there are several densely populated unplanned settlements, e.g. Daraja Mbili and Lemara. Apart from human settlements, there are several industrial and agricultural activities taking place along the river, as indicated in Fig.1 and 2. Also, WSPs and a CW as dominant techniques for EDCs removal were assessed both at effluent and influent points.

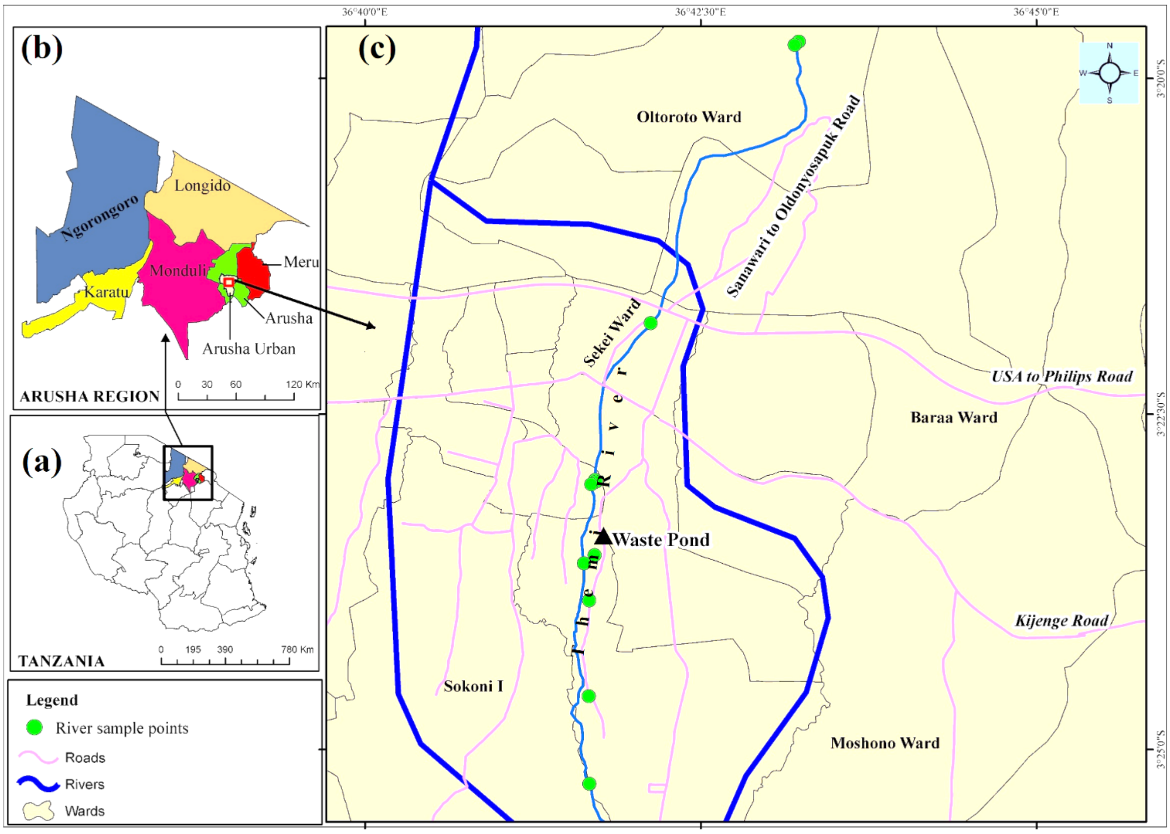

Figure 2. The map of (a) Tanzania, (b) Northeast Tanzania and (c) Arusha city area, the area where water samples were collected. Themi River is indicated by the middle thin blue strip with sampling location in green circles.

3.2 Materials used

The materials used in this study included; progesterone *AccuBind*® ELISA Test System Kit (Lake Forest, CA 92630, USA), and Methanol (99%) of HPLC grade from Sigma Aldrich (Germany) for progesterone extraction. Hydrochloric acid (37%, 1.18 M) (Sigma Aldrich, Germany) for pH adjustment and minimization of microbial degradation in the sample. Distilled water for washing purposes. Solid-phase extraction C-18 cartridges (500 mg, 6 mL) supplied by J & K Scientific (Beijing, China) used for hormone concentration and matrix removal. The n-heptane (99.00%) and acetone (99.80%) used for elution, membrane filter DSO210-4045 used for debris and particle removal were all supplied by Fischer Scientific (Waltham, MA, USA). Multiparameter water quality meter with probe (HI 9829) and pH meter

(GHM 3500 series), HANNA Instruments, Woonsocket, RI, USA) were used to measure pH, conductivity, dissolved oxygen (DO), temperature, and total dissolved solids (TDS). The air vacuum pump Manford 2546C-02 B (Welch, Germany) was used.

3.3 Characteristics of sampling clusters and study design

The study was experimental, whereby the sampling points were selected according to the hypothesis that there is a high probability of being polluted by progesterone. For the Themi River, the study was divided into upstream, midstream and downstream. Upstream was an area with low probability of progesterone pollution (source of Themi River). Midstream involves different sampling points namely: 100 meters before the river joining the WSP effluents; an end where the stream and effluent meet; pure livestock effluents and the location where livestock effluent enters the stream. Agricultural runoff toward the river and congested human settlements to assess progesterone presence of domestic origin. Finally, downstream of the river, where few anthropogenic activities are conducted as described in Table 1.

A total of 18 sampling points was established with a total of 36 samples in which grab sampling was used. For the WSPs and constructed wetland, a total of 26 samples were collected including the influent and effluent of WSPs and constructed wetland compartments. Different sampling points were marked with a Global Positioning System (GPS) and plotted, as indicated in (Fig.1 and 2). Physico-chemical parameters such as pH, temperature, DO, and electroconductivity were recorded *in-situ* by using PalinTest© multiparameter for each sampling point. After pretreatment and solid-phase extraction, the samples were quantified by using enzyme-linked immunosorbent assay (ELISA) competitive technique and all areas with progesterone EDCs were mapped using ArcGIS software. Moreover, the efficiencies of WSPs and selected constructed wetland in removing EDCs were assessed.

A summary of the important environmental, social and economic characteristics of the sampling area presented in Table 1

Table 1. Summary of the environmental and socio-economic characteristics of the sampling area.

Sampling area category	Characteristics
Upstream section	• River source location • Sparse vegetable gardens e.g. celery • Less human settlement • No stabilization ponds • Less industrial development/ activities • No livestock keeping
Midstream section	• More human settlement • Chemical-intensive, horticultural activities • More industrial activities • Livestock keeping • Location of WSPs • Shallowest riverbed
Downstream section	• Rain fed, smallholder agricultural areas • Less industrial activities (beyond industrial zone) • Less human settlement • Located post-WSPs • Discharge of spring water in the river • A deeper riverbed

3.4 Sample collection and preparation

Water samples were collected for five consecutive days at the end of March 2019. The days were sunny with water temperature ranging between 25.8 - 26.8 °C. One-litre capacity bottles were used to collect water samples taken 20 cm below the water surface. For collection of WSPs and CW effluent and influent samples, a water sampler was used. The collected samples were adjusted to pH 3.0 by adding HCl. Then the samples were stored in a cool-box packed with the ice packs to keep the samples cold, below 4.0 °C before being transported to the NM-AIST laboratory for further analysis. Samples were analyzed within ten days after sampling while stored at –20.0 °C. Before extraction, wastewater samples were filtered twice using GF/C and membrane filter of pore size 0.45 µm to remove suspended solids and debris, while the Themi River samples were filtered once by using a membrane filter 0.45 µm.

3.5 Extraction of hormones

After filtration, solid phase extraction (SPE) carried out according to Hansen *et al.* (2011) protocol with some modifications done in the laboratory as described in Fig. 3. The water samples were thawed at room temperature before commencing the extraction process. The solid phase extraction was conducted using C-18 cartridges (500.0 mg, 6.0 mL) facilitated by a Manifold© vacuum pump. The C-18 cartridges conditioned with 2×3.0 mL n-heptane, 3.0 mL acetone, and finally, with 3.0 mL of distilled water adjusted to a pH of 3.0 to remove impurities. The samples were then loaded into the extraction system supported by a Manifold© pump at the rate of 6.0 mL/min in which the particles were trapped by a glass fibre filter. The cartridges were washed with a 1.0 mL methanol at a rate of 2.0 mL/min to remove hydrophobic substances. After extraction, the cartridges were dried out in air using a vacuum manifold for about 30.0 min and then eluted using a mixture of 10.0 mL of heptane and acetone (65:35). The elutes were dried in air at 30.0 °C, and then reconstituted in 5.0 mL methanol and subsequently stored at –20.0 °C for ELISA analysis.

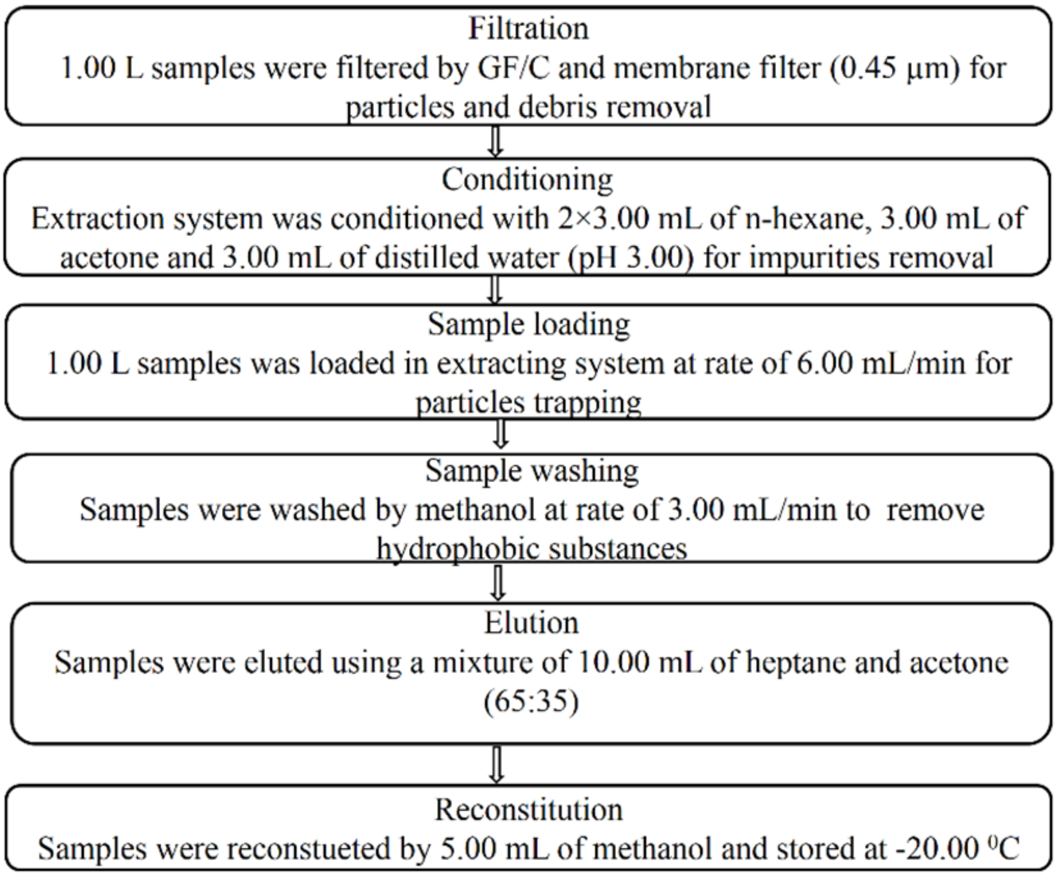

Figure 3. Processes in solid phase extraction (SPE) of water samples.

3.6 ELISA analysis

Before analysis, samples and ELISA kits were thawed at room temperature between 20.0 and 27.0 °C for 30 min according to the manufacturer's recommendations. For ELISA techniques a buffer solution was prepared by diluting the content of wash solution to 1000.0 mL distilled water and stored at a temperature between 2 .0 and 8.0 °C. All microplates' wells for each reference calibrators, samples, and control were assayed in duplicate. Seven referenced standards (1–7) and 76 water samples (8–84) of 25 µL pipetted into assigned wells. Progesterone enzymes reagent of 50.0 µL was added to all wells and swirled for 10.00–20.00 s, then covered and incubated for 60.0 min at room temperature. Then the microplates were decanted and then dried by using a washing machine, then 350.0 µL of wash buffer was added three times. Then 100.0 µL of substrate solution was added to all wells at the same time to minimise time differences between wells and then incubated at room temperature for 20.0 min. Lastly, 50 µL of stop solution was added in each well at the same time and gently mixed for 15.0 –20.0 s. The quantification of the sample was done within 15.0 min after the reaction stopped in which sample absorbance measured with a microplate's reader at 450 nm.

3.7 Quantitative data analysis

The concentration of standards (0, 0.3, 2, 5, 15, 30 and 60 ng/mL) were transformed into common logarithm in which concentration was plotted against absorbance to obtain a linear calibration curve. The linear equation derived from the curve was used to interpolate the progesterone concentration in each sample.

3.8 Statistical analysis

For statistics, GenStat 15^{th} edition (VSNi, Rothamsted, England) and OriginPro 9 (Originlab Corporation, Wellesley Hills, United States) were used for statistical analyses of both descriptive and inferential results. For descriptive statistics, mean and standard deviation were calculated. For Inferential statistics one-way ANOVA with post hoc Turkeys involved for multiple comparisons of progesterone levels between sampling clusters. The significant difference between groups was reported as at $P < 0.05$. All graphs were drawn by using OriginPro 9 software.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Progesterone levels

4.1.1 Progesterone standard curve

Progesterone standard curve was drawn by taking common logarithms of standard concentrations against absorbance to obtain linear curves, as shown in Fig.4. The R^2 value obtained was 0.99. The linear equation derived was used to calculate the concentration of progesterone based on their corresponding absorbance.

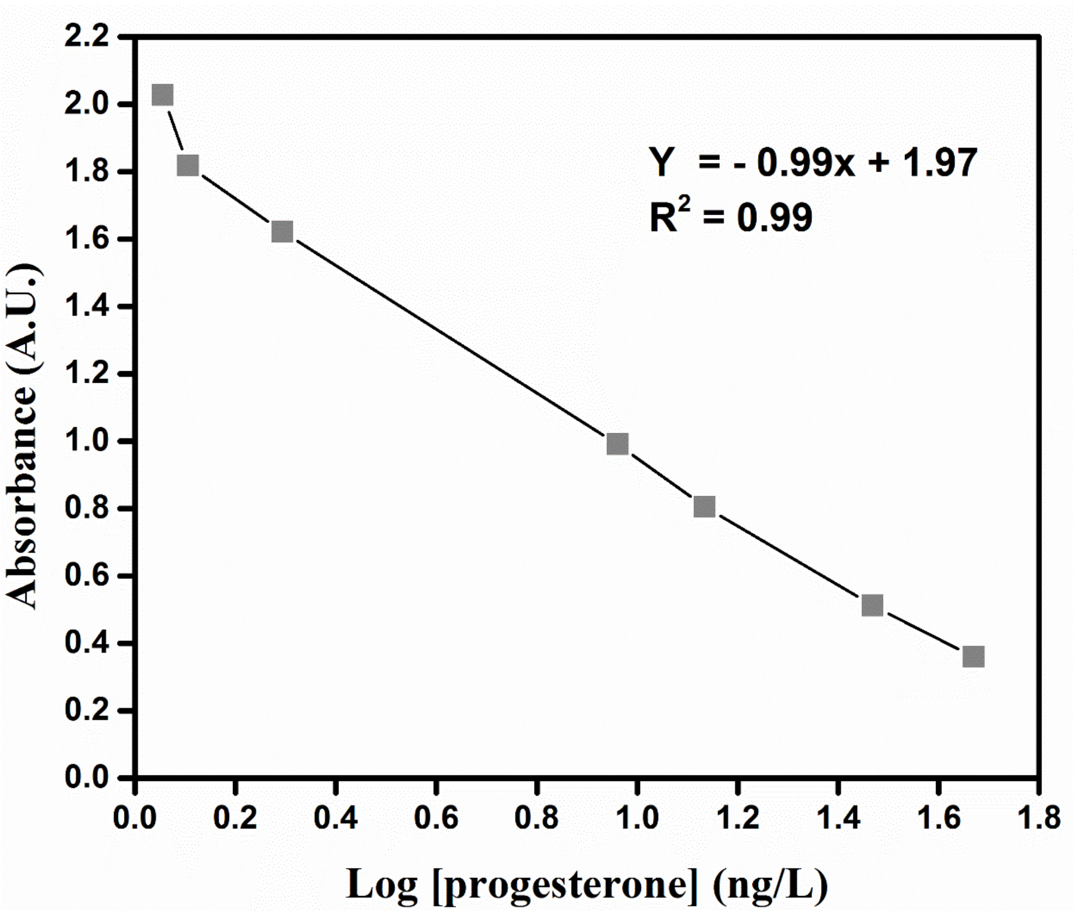

Figure 4. Dose-response curves for standard concentrations showing the resulting linear equation and R^2 values.

4.1.2 Overall patterns

Table 2 and Fig. 5 show the mean concentrations of progesterone in the Themi River. The results indicate that the midstream section progesterone levels were significantly higher ($P < 0.05$) than those at the upstream and downstream segments. Comparison between the upstream and downstream samples shows that the downstream was more polluted by progesterone than the upstream ($P < 0.05$). This indicates that many anthropogenic activities taking place at the midstream section of the river makes it more prone to pollution. Overall, the levels of progesterone in the current study are relatively low compared to those reported in the literature (Jenkins *et al.*, 2003; Chang *et al.*, 2009; Payus *et al.*, 2016). Also, the level of progesterone in this study is higher than those who reported by other studies (Pauwels *et al.*, 2008; Chang *et al.*, 2011; Manickum & John, 2014). This level is high enough to cause effects to aquatic organisms. Some epidemiological research has shown that environmental impacts of EDCs can be revealed even after a short period of exposure depending on the concentration of EDCs (DeQuattro *et al.*, 2012). According to DeQuattro *et al.* (2012), organisms can be affected by progesterone at concentrations ranging from 100 to 1000 ng/L within seven to eleven days.

Table 2. Levels of progesterone (ng/L) in Themi River, Arusha WSP, and livestock effluent.

	Themi River sections				Arusha WSPs		
Statistic	Upstream	Midstream	Downstream	Overall	Influent	Effluent	LEEP*
n	4	8	3	15	5	5	3
Minimum	20.40	68.60	44.90	20.40	250.20	215.00	624.00
Maximum	38.00	439.00	56.50	439.00	2488.00	1936.00	1340.00
Mean	23.40	166.60	48.50	120.30	862.90	667.00	904.00
SD (±)	1.20-5.60	0.90-19.20	0.80-3.40	0.5017.20	11.0-42.0	4.20-77.0	7.1-24.6

*LEEP = Livestock effluent entry point

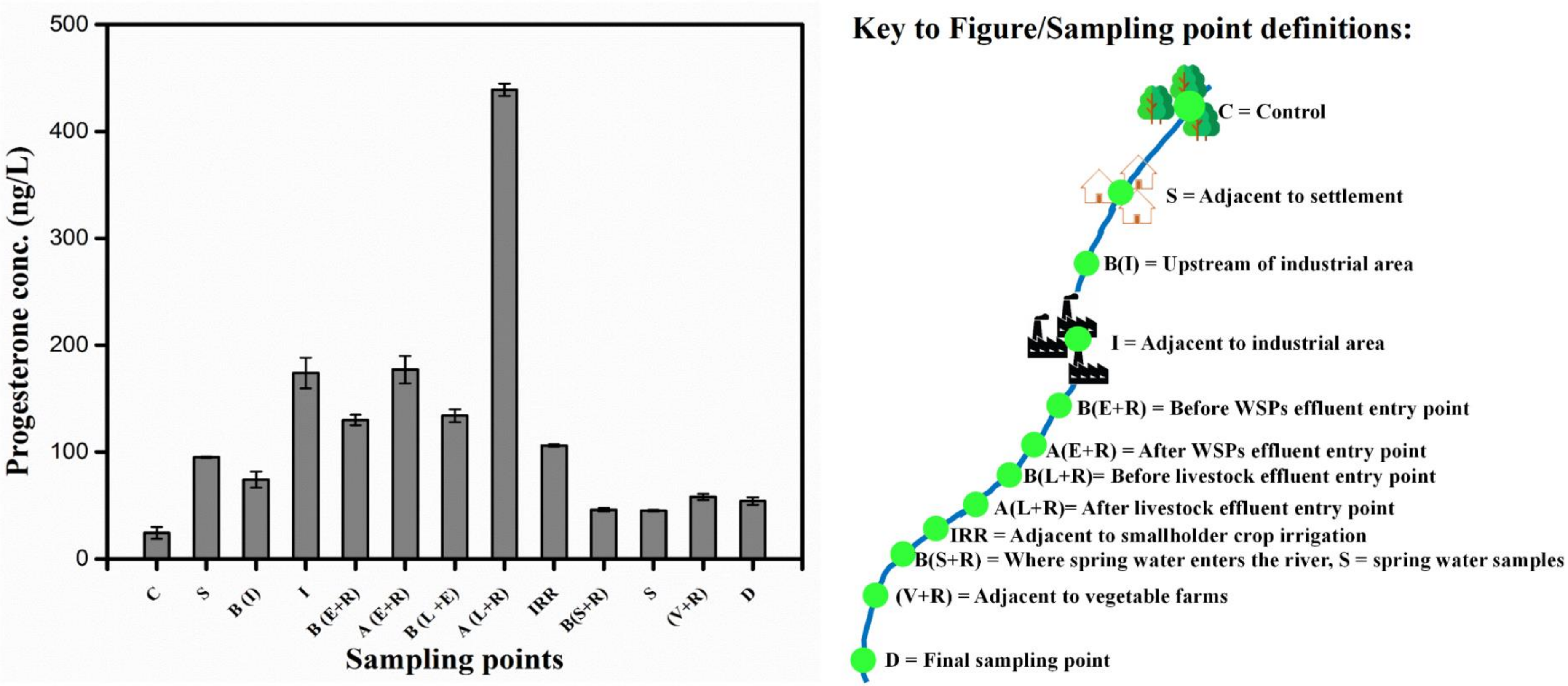

Figure 5. Progesterone concentrations at various sampling points in Themi River water samples. Definitions of the x-axis labels are given on the right.

Table 3. Mean (± SD) progesterone concentration across the study area.

Sampling point	Progesterone concentration (ng/L)
Control area	24.3 ± 5.6
Settlement	95.0 ± 0.6
Before industrial area	74.0 ± 7.6
Industrial area	174.0 ± 14.4
Before effluents discharged in the river	130.0 ± 4.8
After effluents discharged in the river	177.0 ± 12.9
Before livestock effluent discharged in the river	134.0 ± 6.1
After livestock effluent discharged in the river	439.0 ± 5.8
Irrigation effluents	106.0 ± 1.1
Before spring water discharged in the river	46.0 ± 1.6
Springwater discharged in the river	45.0 ± 0.9
The effluent at which vegetation planted in the river	58.0 ± 2.8
Downstream	54.0 ± 3.5

n = 2 for each sample point

4.1.3 Upstream progesterone levels

Overall, the lowest level of progesterone was found upstream in the samples collected close to the river source with an average of 23.40 ng/L, as indicated in Fig. 6 and Table 2. It was expected that at the river source, there would be very little to no progesterone levels, but results from this study indicate, this was not the case. Even at the river source, there were measurable amounts of progesterone. Table 3 indicates the mean values of progesterone from one point to another along Themi River. Progesterone at the river source was probably due to the degradation of natural products in leafy and other plant materials (Jenkins *et al.*, 2003). Moreover, it was observed that there were small plots of celery farming in areas near the river source. This small-scale vegetable farming probably contributed to the levels of progesterone found in the water samples. Also, informal discussion with some farmers in the study area revealed that although the use of synthetic fertilizers in vegetable production in the area is prohibited, it is not uncommon for some farmers to smuggle fertilizers and other chemicals into their plots to boost smallholding horticultural production.

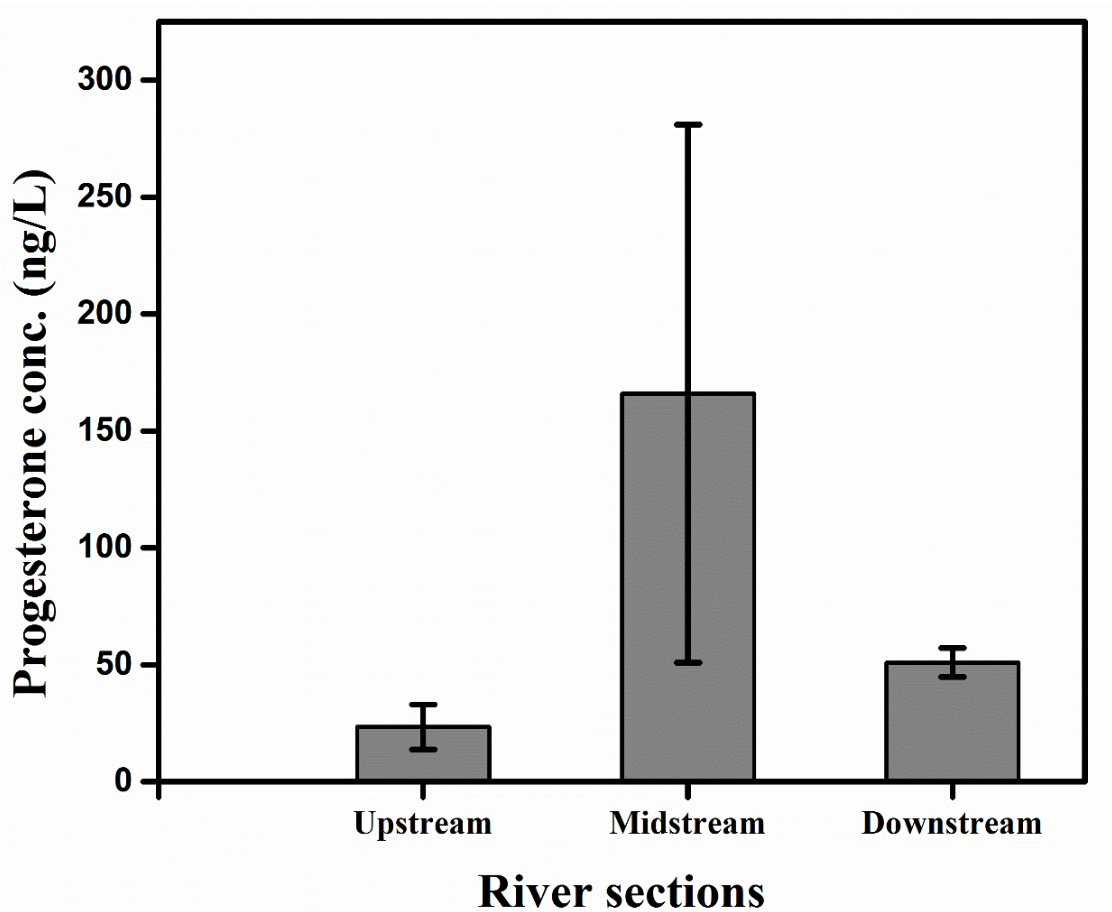

Figure 6. Overall variations in progesterone levels from upstream to downstream sections of the Themi River.

4.1.4 Midstream progesterone levels

The levels of progesterone in the midstream portion of the river were higher than those found in the upstream and downstream parts (Fig. 6). The mean progesterone concentration obtained at midstream was 166.0 ± 115.0 ng/L. The high standard deviation value was probably due to many varied sources of progesterone at the midstream section of the river. High levels of progesterone in the midstream section of the river were possibly attributable to the various anthropogenic activities taking place in that section (see Table 1) (Chang *et al.*, 2009; Liu *et al.*, 2012).

At midstream, the river also received effluent from WSPs with the concentration of 177.0 ng/L at the point where WSPs effluent entered the river (Table 2). However, the amount of progesterone in the receiving waters was comparable to those found in previous studies such as 5-199 ng/L (Kolpin *et al.*, 2002; Chang *et al.*, 2011). Compared to other identified sources, the WSPs contribute appreciably on the levels of progesterone in the Themi River.

Results of this study indicate the highest progesterone concentration (439.0 ± 0.2 ng/L) at the point where effluents from livestock keeping entered the river. Some studies conducted elsewhere have also found elevated amounts of hormonal pollutants at locations where effluents from livestock enter the receiving waters (DeQuattro *et al.*, 2012; Liu *et al.*, 2012; Havens *et al.*, 2014). The mean progesterone concentration obtained from pure livestock effluent was 908.0 ng/L, which is two times higher than the levels found at the entry point into the river. This decrease in hormonal concentrations may be attributable to hormonal degradation by sunlight, sedimentation or infiltration into the soil as water flows towards the river. The high levels of progesterone in livestock effluents may also be indicative of hormonal chemicals used in livestock production in the area. Hormonal supplements are probably supplied to livestock as growth promoters, weight and yield improvers, and immunity enhancers which poses a threat to animal, human and environmental health. If released in high doses, hormonal pollutants may easily find their way into the air, soil, surface water and groundwater causing contamination in the fate media (Chang *et al.*, 2009; Kabir *et al.*, 2015). The direct discharge of livestock effluent into the river should be discouraged since its contribution to the progesterone pollutant is very high compared to other sources.

4.1.5 Downstream progesterone levels

The level of progesterone at downstream was lower than midstream but slightly higher than upstream, as indicated in Fig. 5 and 6. This would not usually be the case as higher contaminant levels are usually expected in downstream samples. Low levels of progesterone downstream were probably due to the dilution effect due to the presence of springs in the downstream area. Springwater entered and mixed with Themi River at various points in the downstream section of the river. Low levels of progesterone in the downstream section might also have been due to the metabolic activities of some bacteria which transformed progesterone into other androgens such as 17α-Hydroxyprogesterone (17α-OHP), androstenedione, testosterone, estrone, and estradiol (Jenkins *et al.*, 2004) as shown in Fig. 7. Furthermore, EDCs were probably reduced due to different natural processes taking place in the water, including biodegradation, sorption and photolysis. Moreover, low concentration of progesterone may be attributed to various microbial activities such as strains of fungi called *Cyanobacteria Microchaeta tenera* and two microalgae namely *Scenedesmus obliquus* and *Chlorella pyrenoidosa* which may have converted progesterone into other by-products (Lu *et al.*, 2009; Peng *et al.*, 2014; Ojoghoro, Chaudhary, Campo, Sumpter & Scrimshaw, 2017).

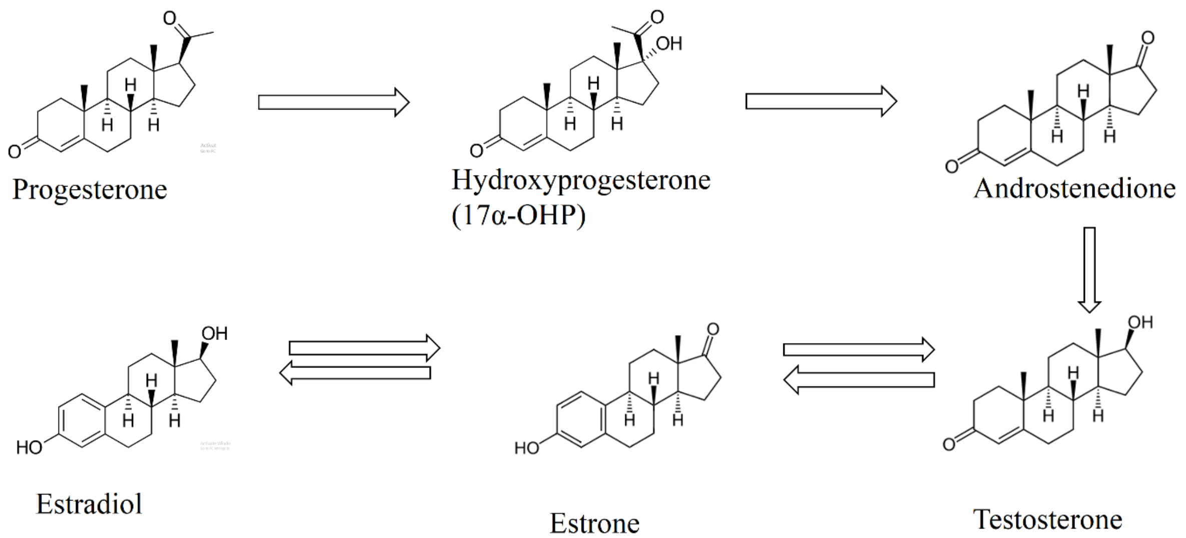

Figure 7. Conversion of progesterone into other androgens (*Source*: Ojoghoro, Chaudhary, Campo, Sumpter & Scrimshaw, 2017).

4.2 Spatial distribution of progesterone along Themi River

Fig. 8 shows the general distribution of progesterone along the Themi River. According to field observations during the course of the present study, progesterone may be distributed differently throughout the river depending on the probable sources. The concentration of progesterone increased with the increase in anthropogenic activities performed around a sampling point in question. Progesterone concentration was low at upstream and downstream points but significantly higher at midstream. Unplanned settlements with poor sanitation and waste management facilities may have played a role in discharging untreated waters in the Themi River. Also, direct discharge of effluent from car washing and poorly managed health facilities would probably be another point source of the contaminant. It is hypothesized, therefore, in the present study that progesterone concentration variations along the Themi River may be attributable to variations in anthropogenic activities. Further studies to ascertain the actual sources of progesterone are, however, recommended.

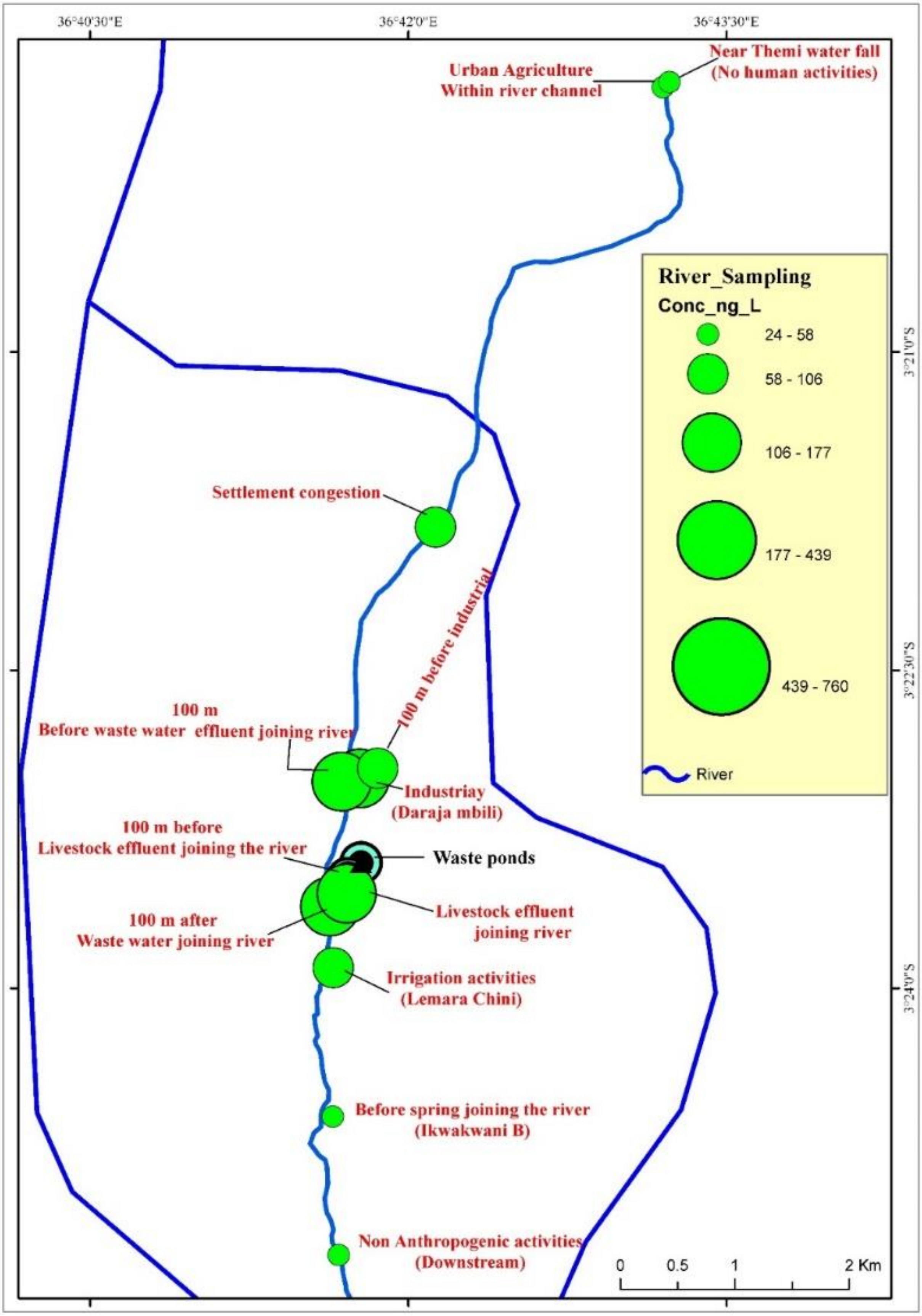

Figure 8. Distribution of progesterone along Themi River. Concentration is in ng/L.

4.3 Physical parameters vs. progesterone levels and distributions

The present study shows that physical environmental parameters such as temperature, dissolved oxygen, electric conductivity, pH, total dissolved solids and salinity do not affect the levels and distribution of progesterone in WSPs and receiving waters (Tables 4 and 5). There is no significant correlation between most of the physical parameters and progesterone levels. However, there is strong correlation between WSPs outlet DO and outlet progesterone concentration. It seems that as dissolved oxygen increases at the pond outlets, processes that favor progesterone also increase. For data from the Themi River, the highest concentration of DO (40.8 mg/L) was found at the control area and at the point where spring water enters the river (41.2 mg/L) and at both points the amount of progesterone was the lowest. Interestingly, at the point where livestock effluents joined the river, the DO concentration was as low as 27.80 mg/L whereas pure livestock effluent had almost zero DO, these two points had the highest amount of progesterone. According to Koumaki, Mamais &Noutsopoulos (2018), high DO levels lead to faster EDCs degradation and biotransformation, which may have led to lower concentrations of progesterone in these aquatic environs.

Table 4 Levels for physico-chemical parameters in water samples collected from the Arusha WSPs.

WSP component	Sampling point	[Progesterone] (ng/L)	Temperature (°C)	pH	DO (mg/L)	EC (NS/CM)	TDS (mg/L)	Salinity (PSU)
Anaerobic	Inlet	2487	26.4	7.3	38.9	6997.0	4390.0	0.4
	Outlet	1932	26.4	7.3	39.8	7759.0	5044.0	4.3
Facultative 1	Inlet	791	25.7	7.2	39.0	7469.0	4784.0	3.8
	Outlet	552	25.3	7.2	39.1	3881.0	2523.0	2.0
Facultative 2	Inlet	418	26.6	7.4	38.9	5587.0	3630.0	3.0
	Outlet	403	26.1	7.2	39.2	5540.0	3609.0	3.0
Maturation 1	Inlet	365	25.1	7.4	39.8	5572.0	3636.0	3.0
	Outlet	269	25.0	7.2	40.1	3847.0	2501.0	2.0
Maturation 2	Inlet	253	24.6	7.1	40.3	5045.0	3068.0	2.4
	Outlet	216	26.6	7.0	38.8	6459.0	4206.0	3.5

n = 2 for each sampling point

Table 5. Levels physico-chemical parameters of water samples collected from Themi River.

Sampling point	Progesterone conc. (ng/L)	Temperature (^{0}C)	pH	DO (mg/L)	EC (µS/cm	TDS (mg/L)	Salinity (PSU)
Control area	24.3	24.3	6.3	40.8	484	314	0.2
Settlement areas	95	25.5	6.3	40.1	561	365	0.2
Before industrial areas	74	25.3	7.1	39.6	6449	4227	3.6
Industrial area	174	25.8	6.3	39.8	942	612	0.1
Before effluents discharged in the river	130	25.2	7.2	38.2	645	324	0.3
After effluents discharged in the river	177	26.7	7	37.3	3551	2341	1.9
Before livestock effluent discharged in the river	134	25.7	7.4	38.2	3420	2420	1.3
Livestock effluent	760	25.5	11.9	0	6556	6249	--
After livestock effluent discharged in the river	439	25.3	7.6	27.8	3249	2345	0.1
Irrigation effluents	106	26.5	7	37.2	4625	3006	2.5
Before spring water discharged in the river	46	25.6	6.8	39.4	2659	1922	1.5
Spring water discharged in the river	45	23.4	6.6	41.2	3225	2095	1.8
Effluent at which vegetation planted in the river	58	24.2	7.8	39.2	3278	2358	1.3
Downstream	54	25.9	6.8	40.8	2993	1944	1.3

n = 2 for each sampling point

4.3 Progesterone removal by WSPs and CW processes

This study assessed the efficiency of a constructed wetland (CW) at NM-AIST in removing progesterone. CW processes are nowadays regarded as one among the most effective techniques in eliminating environmental pollutants. CW systems for wastewater treatment include the use of engineered structures that are built to utilize natural processes. These systems exploit the natural processes comprising of vegetation, soils, and their associated microbial assemblages for the treatment of wastewater (Kaseva, 2004; Zhang, 2012; Qiang *et al.*, 2013). However, CW technology is not popular in most developing countries due to lack of knowledge on how to construct system and unawareness on the advantages of using CW systems for removing pollutants (Kaseva, 2004).

The NM-AIST CW has two compartments with a horizontal flow in which wastewater is fed into the inlet continues and comes out at the outlet. Wastewater flows below the bed surface in a more or less horizontal path until it reaches the outlet zone. The wastewater flows through a porous medium. As the water passes through the medium, it will contact different zones such as aerobic, anaerobic, anoxic and filtration zones. Cell 1 of the CW (Fig. 9) is configured to include baffles, which makes wastewater to take more extended pathway resulting in more contact with the rhizomes and micro-aerobic zones (Vymazal *et al.*, 2015).Wetland cells have a hydraulic retention time of 0.72 days within a 0.5 m depth of wetted portions.

Results for the levels of progesterone as wastewater passes through different compartments of the NM-AIST CW are indicated in Fig 10. The CW inlet received 695 ± 113 ng/L of progesterone which enters into the wetland and is directed to the filtration bed where Macrophytes perform degradation and biotransformation of different pollutants. The concentration of EDCs detected at the end of each cell of the planted portion was 603 ± 123 and 1742 ± 79 for Cell 1 and Cell 2, respectively. The concentration of progesterone decreased in Cell 1, which had low oxygen and maintained anaerobic condition, which may cause the rate of biodegradation and biotransformation to be low. However, this study found that instead of decreasing, progesterone in Cell 1 increased. The reasons for the increase in progesterone concentration is not well known. Probably the progesterone increased due to the presence of microbial biotransformation of phytosterols into progesterone, which was later transformed into other androgens (Jenkins *et al.*, 2004). Free phytosterols usually originate from vegetable oil and its products such as margarine (Qiang *et al.*, 2013).

After passing through Cell 1 and 2, wastewater goes through chambers that have macrophytic plants before being allowed to empty into the receiving environment. The final discharged effluent into the receiving environment was found to contain negligible amounts of progesterone. This was probably due to high retention time in the macrophytic chambers coupled with microbial and photolytic degradation of progesterone. A study on the processes involved in removal of EDCs from wastewater at different stages in a wetland system is thus recommended.

In the present study the WSPs' progesterone levels were also assessed. The results of progesterone levels for different sections of the Arusha WSPs are indicated in Fig 11. The highest concentrations were found in anaerobic ponds' influent, whereas the lowest levels were found in the maturation ponds effluents The Arusha WSPs receive a maximum of 2488.00 ± 1.30 ng/L of progesterone at influent with a mean concentration of 862.90 ng/L. It releases into the Themi River a minimum of 215.00 ± 1.40 ng/L progesterone in the effluent. Comparable results have been stated by other authors who evaluated the levels of EDCs elsewhere (Chang *et al.*, 2011; Manickum & John, 2014; Madikizela *et al.*, 2017; Zhang *et al.*, 2017). Other researchers, e.g. Payus *et al.* (2016), however, reported higher levels of progesterone in waters associated with WSPs. The relatively low levels of progesterone in the WSPs found in the present study may be due to natural attenuation of progesterone. Usually, progesterone undergoes biodegradation but can still be detected in the effluents of both industrial and municipal wastewater and even in the receiving rivers. Receiving rivers have been reported to have high levels of progesterone compared to other streams which do not receive wastewater effluents (Corrales *et al.*, 2015). WSPs and many other wastewater treatment systems cannot adequately remove progesterone which causes a high level of progesterone in the receiving environment.

Even though different types of wastewater passed through the Arusha WSPs and the NM-AIST CW, results from the present study indicate that the capacity of a CW system to remove progesterone was higher than that of the WSPs. This is in agreement with other researchers e.g. Qiang *et al.* (2013).

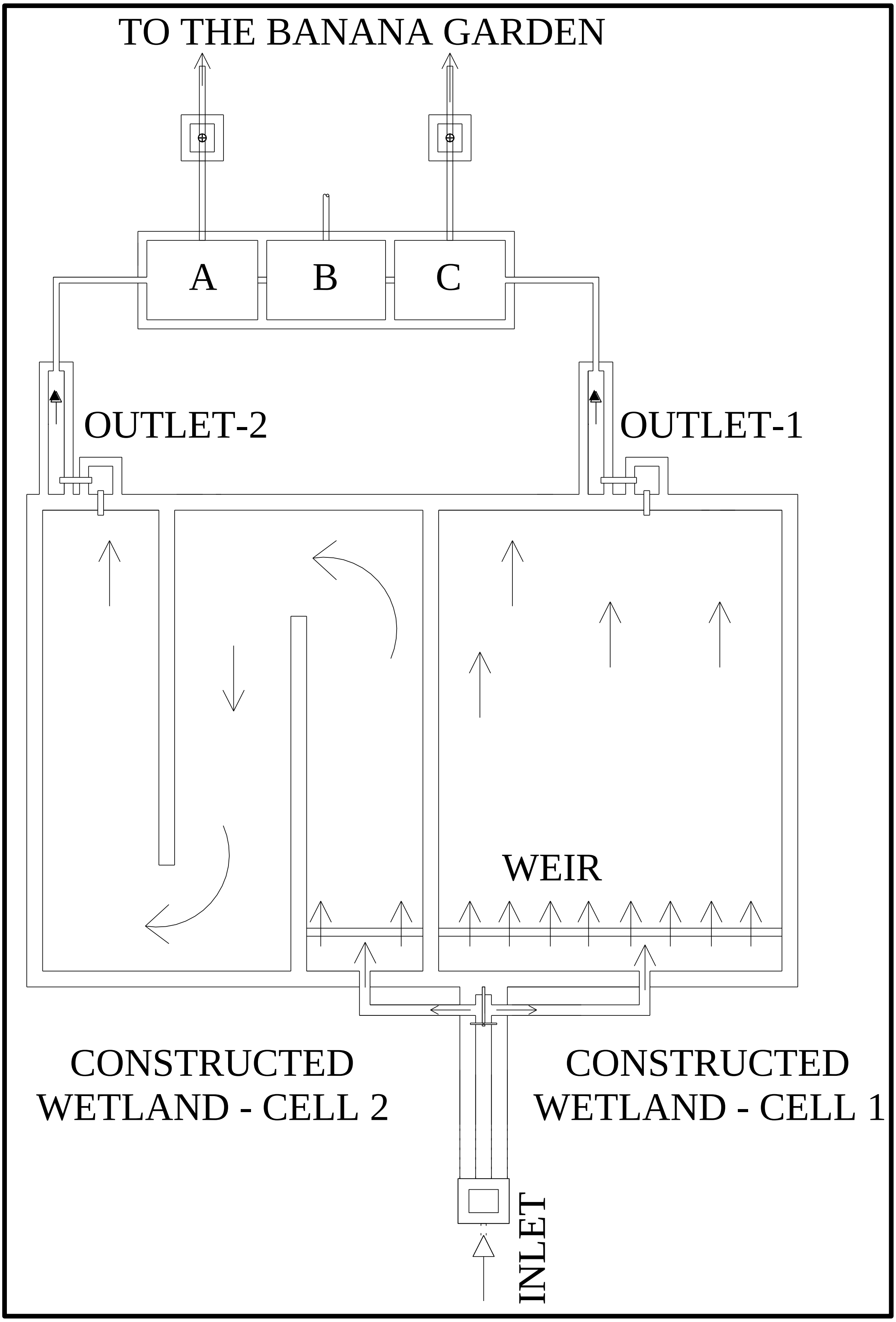

Figure 9. Schematic diagram of constructed wetland at NM-AIST. A is a receiving pond from Cell 2, B is a combined pond and C is a receiving pond from Cell 1.

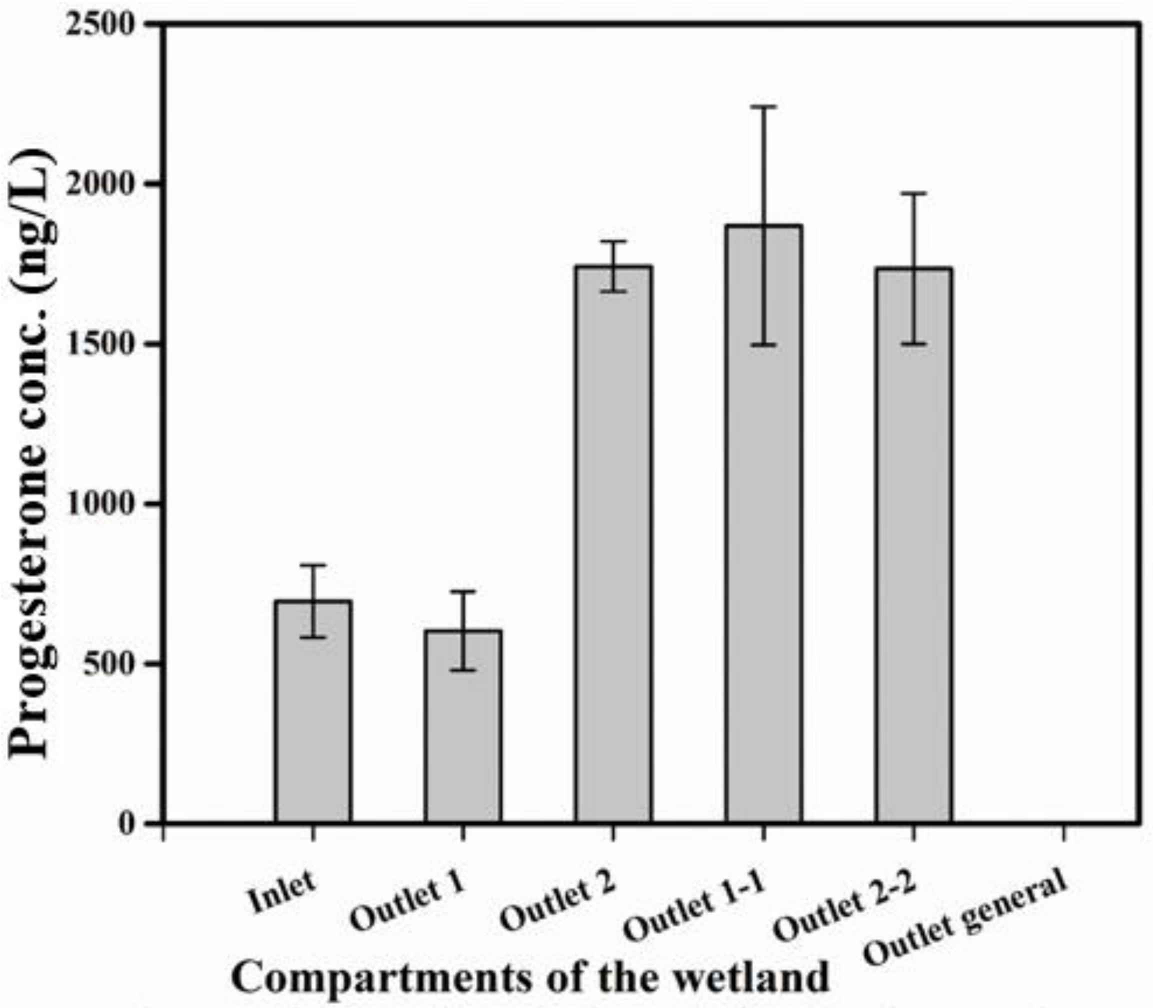

Figure 10. Variation of progesterone concentration in the different compartments of the CW.

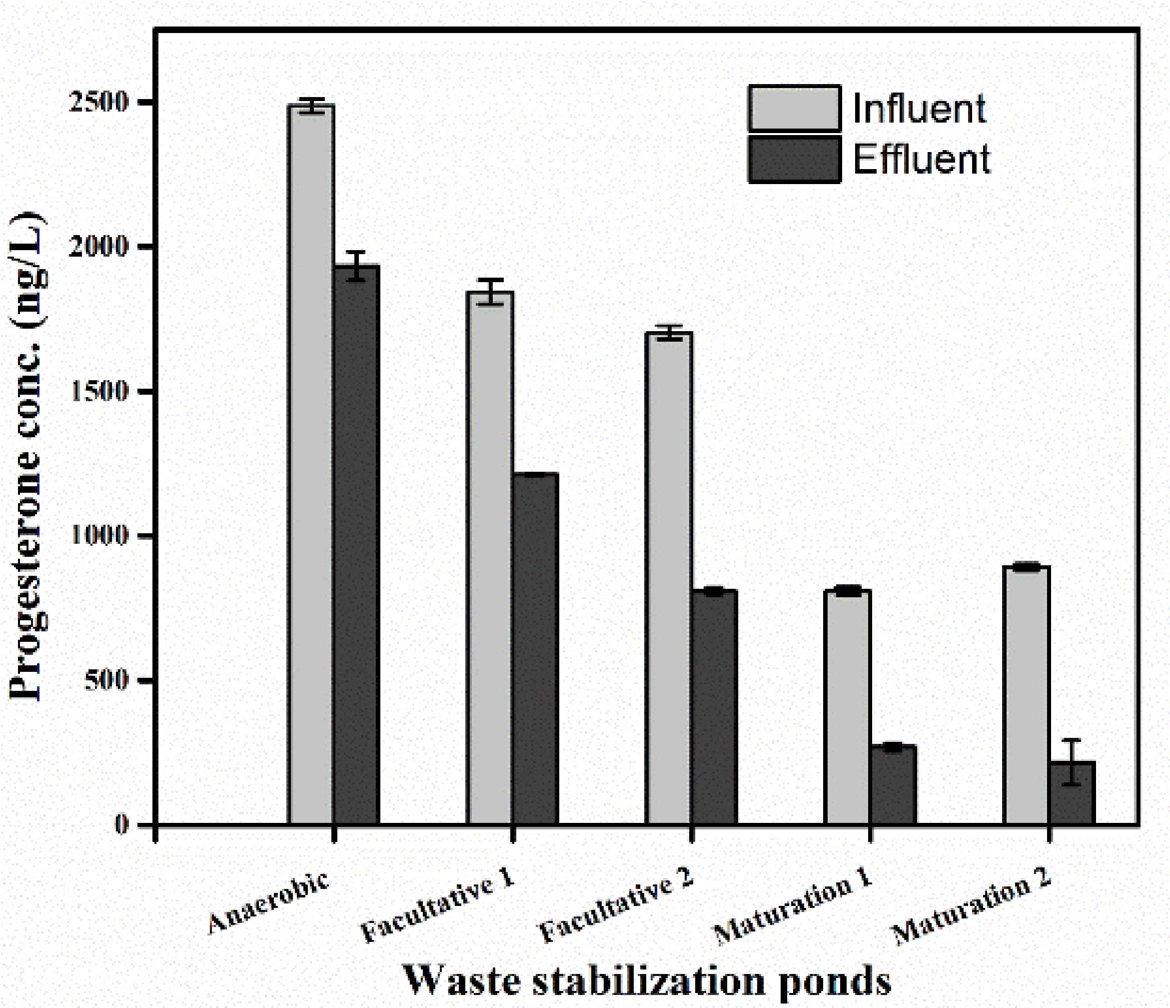

Figure 11. General trends in progesterone concentrations in the Arusha municipal WSPs.

4.3.1 Factors affecting WSPs progesterone removal efficiency

It is also essential to note that usually, WSPs receive influents that have a combination of multiple micropollutants. The micropollutants typically might be converted into other forms. This transformation process may result in reduced levels of the primary pollutants. Moreover, the level of progesterone at effluent mostly depends on the removal efficiency of the WSPs. High effectiveness in removing progesterone leads to high quality effluent received by surface waters and may also have some influence on the quality of groundwater. In the present study, the progesterone removal efficiency was measured by comparing the amount of progesterone at influent and effluent of each pond, as shown in Equation (1):

$$\text{Removal efficiency}(\%) = \frac{[\text{Progesterone}]_i - [\text{Progesterone}]_e}{[\text{Progesterone}]_i} \times 100 \quad (1)$$

where $[\text{Progesterone}]_i$ stands for the influent progesterone concentration and $[\text{Progesterone}]_e$ stands for the effluent progesterone concentration.

Probably the high removal efficiency of progesterone at maturation ponds was due to rapid biodegradation facilitated by high dissolved oxygen (see Fig. 12). Also, the shallowness of the maturation ponds compared to the anaerobic pond, probably help to increase oxygen transfer, which in turn led to increased progesterone biotransformation and biodegradation (Koumaki *et al.*, 2018).

Moreover, the low removal efficiency of progesterone in the anaerobic pond was probably due to shorter retention time and a hence small degree of biotransformation and biodegradation caused by excessive flowrates into the anaerobic pond. The pond designed for an inflow rate of 86 m^3/day, but it was reported to receive inflow rates of up to 6500 m^3/day (AUWSA, 2015). Therefore, WSPs are too overloaded to perform at their expected standard. The removal efficiency of WSPs may also be influenced by many other natural factors, including temperature, wind speed, sunlight, and rainfall. Moreover, the performance of WSPs may be affected by pond design, reduced maintenance and physical-chemical parameters such as pond surface area, water depth, pH and dissolved oxygen (Msigala *et al.*, 2017).

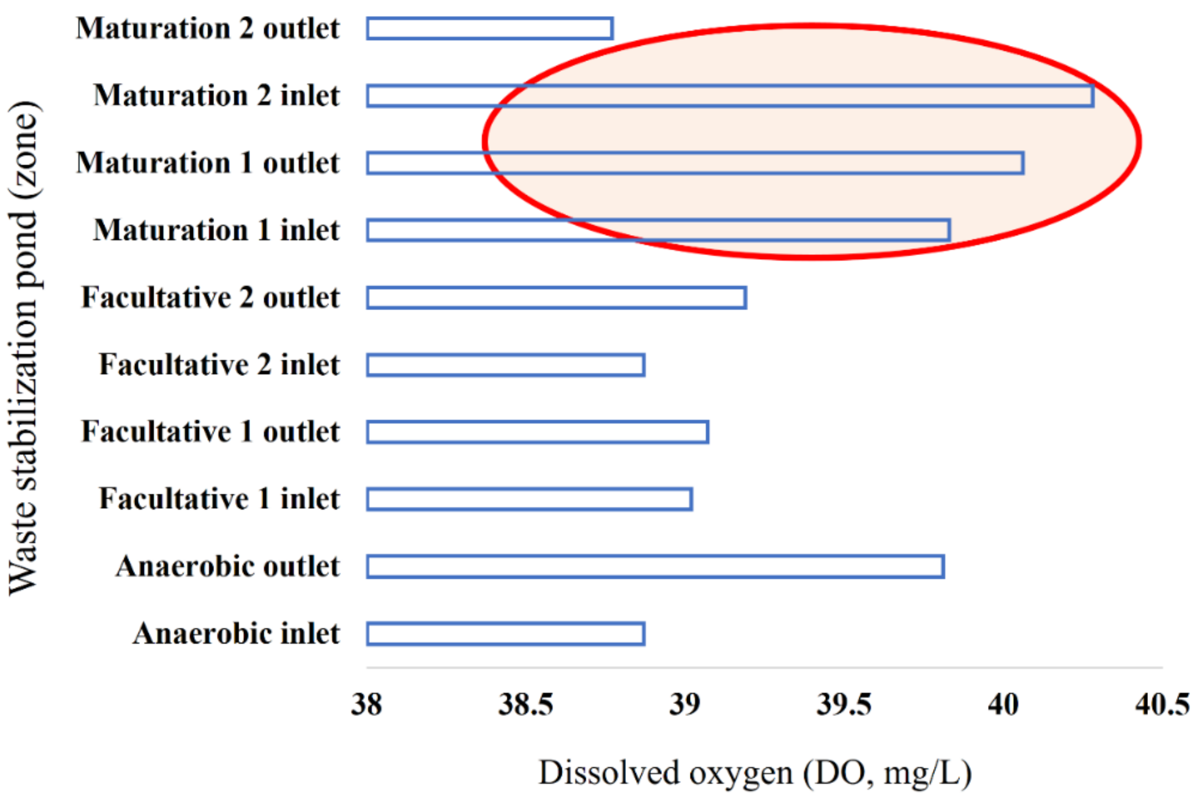

Figure 12. Levels of dissolved oxygen at different zones in the Arusha municipal WSPs. The encircled portion marks the maturation with high DO level.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Results from this study indicate that Themi River and the associated waste stabilization ponds have significant content of progesterone. In the present study, we also measured the levels of progesterone entering and leaving a constructed wetland system at NM-AIST. It is hypothesized from the results of this study that anthropogenic activities such livestock keeping, municipal wastewater effluents, farming and infiltration from untreated sewage may be contributing to elevated levels of progesterone in the analyzed samples. However, the number of samples and study sites were limiting. Thus, results from the present study should be interpreted with caution. Nevertheless, numbers generated as a result of analysis for the present study may be indicative of the prevailing situation during the time of this study.

Highest levels of progesterone were found at the mid-section of Themi River (max. 439 ng/L) compared to the relatively low values that were detected in samples collected at the upstream (max. 38 ng/L) and downstream (max. 56.5 ng/L) ends of the river. It is important to note that overall samples that were associated with livestock effluents had the highest levels of progesterone amounting to 1340 ng/L.

It was also observed that a constructed wetland system was generally more efficient than a series of WSPs in removing progesterone from wastewater. This was from the results of influent progesterone levels compared to effluent progesterone levels for both CW and WSPs systems.

Results obtained in the present study may help to contribute as baseline information for the establishment of guidelines related to standard levels of endocrine pollutants into the receiving waters in Tanzania. However, we would like to recommend the following research areas for further studies:

- Assessment of cost-effective techniques that can be used to sufficiently remove progesterone from water and wastewater.
- Assessment of progesterone and other EDCs in groundwater near constructed wetland systems as well as WSPs.
- Assessment of biodiversity of aquatic organisms along river systems that pass through urban areas and relate this biodiversity data to levels of EDC pollutants.

- Evaluation of the performance of natural wetland versus constructed wetland in removing EDCs.

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